Nanomedicine, Nanotechnology in medicine

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Nanomédecine et nanotechnologies pour la médecine

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Resumé :
La nanomédecine est un domaine relativement récent des sciences et techniques. Sa définition semble parfois imprécise et différentes interprétations sont données à ce terme, notamment entre l'Europe et les Etats-Unis.

En interagissant avec des molécules biologiques donc à l'échelle nanométrique, les nanotechnologies ouvrent un vaste champ d'application et de recherche. Les interactions entre assemblages moléculaires synthétiques ou dispositifs nanométriques et biomolécules peuvent se concevoir tant dans le milieu extracellulaire qu'à l'intérieur des cellules du corps humain. L'échelle nanométrique permet d'exploiter des propriétés physiques différentes de celles observées à l'échelle microscopique telles qu'un rapport surface/volume important par exemple.

Les applications en diagnostique étudiées sont applicables tant pour le diagnostique *in vitro* que pour le diagnostique *in vivo*. *In vitro*, les particules synthétisés et les dispositifs de manipulation ou détection permettent la reconnaissance, la capture, la concentration de biomolécules. *In vivo*, les assemblages moléculaires synthétiques sont essentiellement conçus comme agent de contraste pour l'imagerie.
Un second domaine de la nanomédecine présentant un fort développement est celui des « nanomédicaments » où des nanoparticules synthétiques sont conçues pour la vectorisation et la délivrance de principes actifs pharmaceutiques. Le recours à ces vecteurs permet d'améliorer la biodistribution des médicaments, concentre leur ciblage vers les tissus pathologiques et protège les tissus sains.
Un troisième domaine d'application est celui de la médecine régénérative où les nanotechnologies permettent de concevoir des matériaux biocompatibles destinés au support de croissance des cellules utilisées en thérapie cellulaire.

L'application des nanotechnologies à la médecine soulève des problèmes nouveaux de par certains nouveaux usages qu'elles permettent, par exemple : la puissance nouvelle du diagnostique est elle gérable par le corps médical ? Que signifie traiter un patient sans signe

clinique ? Que devient même la notion de patient en l'absence de signes cliniques ? La nanomédecine peut potentiellement contribuer au développement d'une médecine personnalisée où un diagnostique personnel permettrait de prescrire une thérapie personnalisée efficace.

Il existe dans de nombreux pays un cadre réglementaire existant qui couvre les règles de base de sécurité et d'efficacité des nanotechnologies médicales, qu'il s'agisse d'assemblages moléculaires ou de dispositifs médicaux. Mais un besoin de préciser voire de faire évoluer certains aspects de ces réglementations mobilisent de nombreux experts.

La France est un pays où le développement des nanotechnologies médicales est significatif, à l'instar de l'Allemagne, du Royaume-Uni ou de l'Espagne, en ce qui concerne l'Union Européenne. La communauté scientifique y est active et des partenaires industriels de toute taille y opèrent, même si le transfert de technologies vers l'industrie n'est pas aussi efficace qu'en Amérique du Nord.

Keywords : nanomedicine; nanotechnology; drug delivery; diagnostic; regenerative medicine; theranostic

Mots clés : nanomédecine ; nanotechnologie ; vectorisation de médicaments ; diagnostique ; médecine régénérative ; théranostique.

Abstract:
Nanomedicine is a relatively new field of science and technology. It looks sometimes ill defined and interpretations of that term may vary, especially between Europe and the United States.

By interacting with biological molecules, therefore at nanoscale, nanotechnology opens up a vast field of research and application. Interactions between artificial molecular assemblies or nanodevices and biomolecules can be understood both in the extracellular medium and inside the human cells. Operating at nanoscale allow to exploit physical properties different from those observed at microscale such as the volume/surface ratio.

The investigated diagnostic applications can be considered for in vitro as well as for in vivo diagnosis. In vitro, the synthesized particles and manipulation or detection devices allow for the recognition, capture, and concentration of biomolecules. In vivo, the synthetic molecular assemblies are mainly designed as a contrast agent for imaging.

A second area exhibiting a strong development is the "nanodrugs" where nanoparticles are designed for targeted drug delivery. The use of such carriers improves the drug biodistribution, targeting active molecules to diseased tissues while protecting healthy tissue.

A third area of application is regenerative medicine where nanotechnology allows developing biocompatible materials which support growth of cells used in cell therapy.

The application of nanotechnology to medicine raises new issues because of new uses they allow, for instance: Is the power of these new diagnostics manageable by the medical profession? What means treating a patient without any clinical signs? Nanomedicine can contribute to the development of a personalized medicine both for diagnosis and therapy.

There exists in many countries existing regulatory frameworks addressing the basic rules of safety and effectiveness of nanotechnology based medicine, whether molecular assemblies or medical devices. But there is a need to clarify or to modify these regulations which mobilize many experts.

France is a country where the medical development of nanotechnology is significant, like Germany, the United Kingdom or Spain, as regards the European Union. There is an active scientific community and industrial partners of all sizes, even if the technology transfer to industry is not as effective as in North America.

1 Definition

There is no nanomedicine, there is nanotechnology in medicine. Even if the expression "nanomedicine" has been widely used for a couple of years, it is more proper to refer to "nanotechnology enabled medicine" in different sub-areas of medicine such as diagnostics, therapy or monitoring.

The definition of nanomedicine is slightly different on both sides of the Atlantic Ocean. While the US National Nanotech Initiative clearly refers to the nanoscale, the European Science Foundation and the European Technology Platform on Nanomedicine don't refer to it:

- **The US National Nanotech Initiative**
  Nanotechnology is the understanding and control of matter at dimensions between approximately 1 and 100 nanometres, where unique phenomena enable novel applications. Encompassing nanoscale science, engineering, and technology, nanotechnology involves imaging, measuring, modelling, and manipulating matter at this length scale. Nanomedicine is the application of nanotechnology to medicine.

- **The European Science Foundation**
  The field of nanomedicine is the science and technology of diagnosing, treating and preventing disease and traumatic injury of relieving pain, and of preserving and improving human health, using molecular tools and molecular knowledge of the human body[1].

- **The European Technology Platform on Nanomedicine**
  Nanomedicine is defined as the application of nanotechnology to health. It exploits the improved and often novel physical, chemical, and biological properties of materials at the nanometric scale. Nanomedicine has potential impact on the prevention, early and reliable diagnosis and treatment of diseases[2]

However, nanomedicine is more an academic concept than an industrial one. Medical industry is looking for solutions for patients regardless the involved technology. Therefore nanomedicine is now sometimes classified under "advanced medical technologies" by industry. Nevertheless, the term "nanomedicine" is used in this chapter, for easiness.

The main application areas of nanomedicine are:

- delivery of pharmaceuticals
- *in vitro*, *on vivo* and *in vivo* diagnostics, including imaging
- regenerative medicine
- implanted devices

2 History

The first results related to the development of nanomedicine could be identified in the late 60's at ETH Zurich[3].
The significant technological and industrial development of nanomedicine is more recent, just a couple of decade or so. It has been marked by some large initiatives which paved the way for its development.

In the early 2000's, both the scientist optimism and the challenges which could be addressed by nanotechnology have prompted governmental science and funding organisations to undertake strategic reviews of the current status of nanomedicine, their primary objectives being to assess potential opportunities for better healthcare as well as the risk-benefit analysis of these new technologies, and to determine priorities for future funding.
In early 2003, the European Science Foundation launched its Forward look on nanomedicine. At that time, there was an increasing optimism that nanotechnology applied to medicine would bring significant advances in the diagnosis and treatment of diseases. This first foresight study focused on medical applications of nanosciences and nanotechnology. The Forward Look involved over 100 leading European experts and allowed to determine the current status of the field and to foster debates on strategic policy issues. A policy briefing was published on 23rd February 2005 which summarised the recommendations of the Forward Look.

In June 2003, the UK Government commissioned the Royal Society, the UK national academy of science, and the Royal Academy of Engineering, the UK national academy of engineering, to carry out an independent study of likely developments and investigate whether nanotechnology might raise or is likely to raise new ethical, health and safety or social issues which are not covered by current regulation. The final report was published in July 2004 with 21 recommendations for a sure, safe and responsible development of nanotechnology[4].

In 2004, The Commission of the European Communities released its communication on the European strategy for nanotechnology[5]. In the same time, the High Level Group European Technology Platform Nanomedicine was launched in October 2004 under the initiative of the European Commission. This group of 40 experts from industry, research centres and academia convened to prepare the vision regarding future research priorities in nanomedicine. In September 2005, its Vision Paper and Basis for a Strategic Research Agenda for Nanomedicine was released, as a first step towards setting up a European Technology Platform on Nanomedicine, aiming at strengthening Europe position and improving the quality of life and health care of European citizens.

More recently, in 2007, the European Foundation for Clinical Nanomedicine was established in Basel (Switzerland). This foundation is a non-profit institution aiming at advancing medicine for the benefit of individuals and society through the application of nanosciences. Aiming at prevention, diagnosis, and therapy through nanomedicine as well as at exploration of its implications, the Foundation reaches its goals through support of clinically focussed research and of interaction and information flow between clinicians, researchers, the public, and other stakeholders. The recognition of the large future impact of nanosciences

on medicine and the observed rapid advance of medical applications of nanosciences have been the main reasons for the creation of the Foundation.

On the other side of the Atlantic Ocean, the National Institutes of Health (NIH) released their first roadmap on nanomedicine in 2004[6]. As a follow up, the NIH established in 2005 and 2006 a national network of eight Nanomedicine Development Centres, which served as the intellectual and technological centrepiece of the NIH Nanomedicine Roadmap Initiative. The goal of the Common Fund's Nanomedicine program, as part of the National Health Institutes Nanomedicine Roadmap is to determine how cellular machines operate at the nanoscale level and then use these design principles to develop and engineer new technologies and devices for repairing tissue or preventing and curing disease.
In 2004, the National Cancer Institute (NCI), as part of NIH, launched the Cancer Nanotechnology Plan, a strategic initiative to transform clinical oncology and basic research through the directed application of nanotechnology[7]. The NCI Alliance for Nanotechnology in Cancer is engaged in efforts to harness the power of nanotechnology to radically change the way we diagnose, treat and prevent cancer. This alliance is a comprehensive, systematized initiative encompassing the public and private sectors, designed to accelerate the application of nanotechnology to cancer.

3 Nanotechnology in medicine: the ideal scale

The aim of nanomedicine may be broadly defined as the comprehensive monitoring, control, construction, repair, defence and improvement of all human biological systems, working from the molecular level using engineered devices and nanostructures, ultimately to achieve medical benefits. In this context, nanoscale should be taken to include active components or objects in the size range from one nanometre to hundreds of nanometres. These may be included in a micro-device (that have a macro-interface) or in a biological environment. The focus, however, is always on nano-interactions within the framework of a larger device or directly within a sub-cellular (or cellular) system.
Nanosciences and nanotechnologies imply studying and working with matter at ultra-small scale. One nanometre is one-millionth of a millimetre and a single human hair is around 80,000 nanometres thick. Therefore nanomedicine operates at the same size scale – about 100 nanometres or less – that biological molecules and structures inside living cells operate. A typical protein size lies between 3 to 10 nanometres (nm), while red blood cells are a standard size of about 6000-8000 nm.

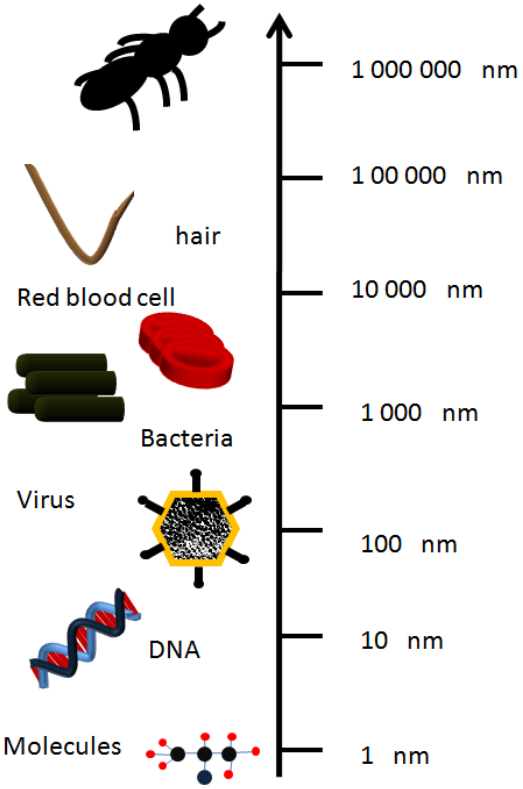

Fig. 1: Scale of size. How small is small

The nanoparticulate systems have a size ranging from a few nanometres, like micelles, to several hundreds of nanometres like liposomes. For instance, drug delivery systems can readily interact with biomolecules located on both the cell surface and inside. Thus nano drug delivery systems can not only transport encapsulated or grafted small chemotherapeutic drugs, with a size of less than a dozens of nanometres, but also deliver them inside cells once they have penetrated them. Such systems can also be decorated with fragments of antibodies on their surface to target specific tissues, thus improving the specificity of the drug delivery.

4 Is the nanoscale really adequate for medical technologies?

Some physical laws are different at nanoscale, and this may be favourable or not for medical applications:

- The surface/volume ratio of particles becomes very large when size decreases, so that nanoparticles have a huge surface suitable for chemical interactions with biomolecules for instance. Moreover, (bio)chemical reaction time are much shorter (it decreases sharply with sample size) and accordingly analytical devices are faster and more sensitive.

- The ultra small size of the sensing part of a (macro- or micro-) analytical device, with nano pillars, nano beads, can be possibly exploited for device miniaturization. Smaller devices offer a lower invasiveness and can even be implanted within the body.

- Another advantage of the ultra miniaturisation of the sensing part lies in the ultra small size of the biological sample required for measurement. This becomes a key feature for analysing rare samples like some biopsies.

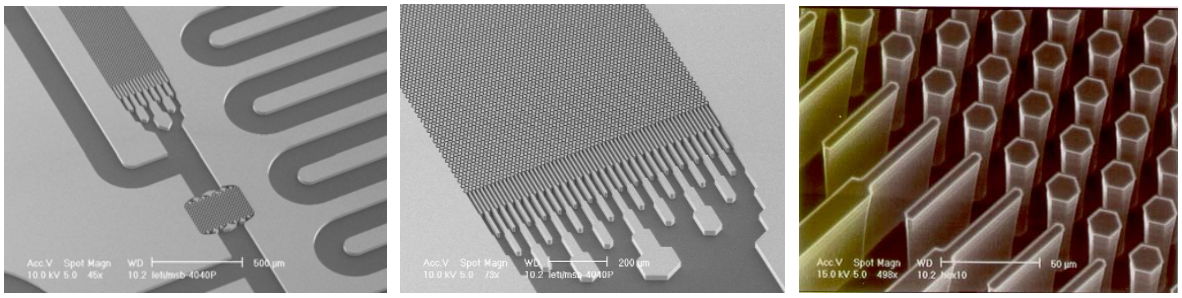

Fig 2: Nanopillars in a BioChipLab microreactor. Credit: CEA-Leti

- On the contrary, measuring low concentrations of biological molecules like some biomarkers in large samples like blood droplets requires preliminary steps for concentrating these molecules. In a general way, bio samples like urine, blood, sweat or tear are micrometric; thus several sample preparation steps are required before analysis.

- Viscosity becomes more effective at the nanoscale. The interaction with capillary walls becomes more important, and the viscosity effect dominates that of gravity. The consequence is that makes nanofluidics more complex than microfluidics.

5 Medical diagnostics

5.1 In vitro diagnostic[8]

In-vitro diagnosis for medical applications has traditionally been a laborious task; blood and other body fluids or tissue samples are sent to a laboratory for an analysis, which could take hours, days or even weeks, depending on the used technique, and is highly labour intensive. The many disadvantages include sample deterioration, cost, lengthy waiting times (even for urgent cases), inaccurate results for small sample quantities, difficulties in integrating parameters obtained by a wide variety of methods and poor standardisation of sample collection. Steadily, miniaturisation, parallelisation and integration of different functions on a single device, based on techniques derived from the electronics industry, have led to the development of a new generation of devices that are smaller, faster and cheaper, do not require special skills, and provide accurate readings. These analytical devices require much smaller samples and will deliver more complete (and more accurate) biological data from a single measurement.
The requirement for smaller samples also means less invasive and less traumatic methods of extraction. Nanotechnology enables further refinement of diagnostic techniques, leading to high throughput screening (to test one sample for numerous diseases, or screen large numbers of samples for one disease) and ultimately point-of-care (POC) diagnostics.

An in-vitro diagnostic tool can be a single biosensor, or an integrated device containing many biosensors. A biosensor is a sensor that contains a biological element, such as an enzyme, capable of recognising and 'signalling' (through some biochemical change) the

presence, activity or concentration of a specific biological molecule in solution. A transducer is used to convert the biochemical signal into a quantifiable signal. Key attributes of biosensors are their specificity and sensitivity.

Techniques derived from the electronics industry have enabled the miniaturisation of biosensors, allowing for smaller samples and highly integrated sensor arrays, which take different measurements in parallel from a single sample. Higher specificity reduces the invasiveness of the diagnostic tools and simultaneously increases significantly their effectiveness in terms of providing biological information such as phenotypes, genotypes or proteomes.

Fig. 3: In check™ lab on chip platform, Credit: ST-Micro

Several complex preparation and analytical steps can be incorporated into 'lab-on-a-chip' devices, which can mix, process and separate fluids, realising sample analysis and identification. Integrated devices can measure tens to thousands of signals from one sample, thus providing the general practitioner or the surgeon with much more complementary data from his patient's sample. Some devices for diagnostics have been developed to measure parts of the genome or proteome using DNA fragments or antibodies as sensing elements and are thus called gene or protein chips. 'Cells–on-chips' use cells as their sensing elements, employed in many cases for pathogen or toxicology screening.
Integrated devices can be used in the early diagnosis of disease and for monitoring the progress of therapy.

New advancements in microfluidics technologies show great promise towards the realisation of a fully integrated device that directly delivers full data for a medical diagnosis from a single sample. Recent developments aim at developing in-vitro diagnostic tools to be used in a standard clinical environment or e.g. as 'point-of-care' devices.

Improvements of specifications of in vitro diagnostic devices thanks to nanotechnologies can be envisaged in two major application areas: Point of care (POC) analysis and central analytical labs. On one hand, miniaturisation, integration and multiplexing would be key features for POC devices. On the other hand, central analytical labs in hospitals need highly automated system with high throughput. However at the moment point of care is not the

main focus of In vitro diagnostic (IVD) industry which concentrates and earns the most money in central clinical labs. However, in the long term the capacity of central lab diagnostics will probably saturate, which will likely result in an increased need for POC diagnostics. The trend towards simple diagnostics tests in the General Practitioner's office and ultimately the home of the patient becomes inevitable. This trend however requires more robust systems, easy to operate without technical training, offering fast response and the delivery of easily analysable data to the practitioner.

5.2 In vivo diagnostic

In-vivo diagnostics refers in general to imaging techniques, but also covers implantable devices. Nanoimaging includes several approaches using techniques for the study of in-vivo molecular events and molecules manipulation. Imaging techniques cover advanced optical imaging and spectroscopy, nuclear imaging with radioactive tracers, magnetic resonance imaging, ultrasound, optical and X-ray imaging, all of which depend on identifying tracers or contrast agents that have been introduced into the body to mark the disease site

The goal of in-vivo diagnostics research is to create highly sensitive, highly reliable detection agents that can also deliver and monitor therapy. This is the 'find, fight and follow' concept of early diagnosis, therapy and therapy control that is encompassed in the concept of theranostics. With this strategy, the tissue of interest can firstly be imaged, using target specific contrast nanostructures. Then, combined with a pharmacologically active agent, the same targeting strategy can be used for applying therapy. Finally, monitoring of treatment effects is possible by sequential imaging.

5.2.1 Imaging

Medical imaging has advanced from a marginal role in healthcare to become an essential diagnostic tool over the last 25 years. Molecular imaging and image guided therapy is now a basic tool for monitoring disease and in developing almost all the applications of in-vivo nanomedicine. Originally, imaging techniques could only detect changes in the appearance of tissues when symptoms were relatively advanced. Later, contrast agents were introduced to more easily identify and map the locus of disease. Today, through the application of nanotechnology, both imaging tools and marker/contrast agents are being dramatically refined towards the end goals of detecting disease as early as possible, eventually at the level of a single cell, and monitoring the therapy effectiveness.

Targeted molecular imaging is important for a wide range of diagnostic purposes, such as the identification of the locus of inflammation, the localization and staging of tumors, the visualisation of vascular structures or specific disease states and the examination of anatomy. It is also important for research on controlled drug release, in assessing drug distributions, and for the early detection of unexpected and potentially dangerous drug accumulations

The convergence of nanotechnology and medical imaging opens the doors to a revolution in molecular imaging (also called nano-imaging) in the foreseeable future, leading to the detection of a single molecule or a single cell in a complex biological environment.

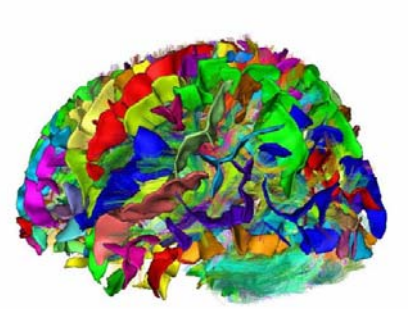

Fig 4. Diffusion Magnetic Resonance Imaging of human brain. Credit: CEA

Current imaging methods can only readily detect cancers once they have caused a visible change to a tissue, by which time thousands of cells will have proliferated and perhaps metastasized. And even when visible, the tumor nature —malignant or benign— and the characteristics that might make it responsive to a particular treatment must be assessed through biopsies. Imagine instead if cancerous or even pre-cancerous cells could somehow be tagged for detection by conventional scanning devices. Two things would be necessary—first something that specifically identifies a cancerous cell and second something that enables it to be seen—and both can be achieved through nanotechnology. For example, antibodies that identify specific receptors found to be over-expressed in cancerous cells can be coated onto nanoparticles such as metal oxides which produce a high contrast signal on Magnetic Resonance Images (MRI) or Computed Tomography (CT) scans. Once inside the body, the antibodies on these nanoparticles will bind selectively to cancerous cells, effectively lighting them up for the scanner. Similarly, gold particles could be used to enhance light scattering for endoscopy techniques like colonoscopies. Nanotechnology will enable the visualization of molecular markers that identify specific stages and types of cancers, allowing physicians to see cells and molecules undetectable through conventional imaging.

5.2.2 Implants, sensors

Implantable Devices for In-vivo Diagnostics

Nanotechnology also has many implications for in vivo diagnostic devices such as the swallowable imaging 'pill' and new endoscopic instruments.

Monitoring of circulating molecules is of great interest for some chronic diseases such as diabetes or AIDS. Continuous, smart measurement of glucose or blood markers of infection constitutes a real market for implantable devices. Miniaturisation for lower invasiveness, combined with surface functionalisation and the 'biologicalisation' of instruments will help increase their acceptance in the body. Autonomous power, self diagnosis, remote control and external transmission of data are other considerations in the development of these devices.

Nanosensors, for example used in catheters, will also provide data to surgeons. Nanoscale entities could identify pathology/defects; and the subsequent removal or correction of lesions by nano-manipulation could also set a future vision.

Nano-harvesting of biomarkers

Researchers attempting to identify disease-related biomarkers in blood face two major problems, each of which the new polymer-based nanoparticles appear to overcome. One issue is that two proteins—albumin and immunoglobulin—account for 90 percent of the molecules in blood, whereas any potential biomarkers are likely to be present at only trace

levels. Furthermore, many blood-borne molecules adhere to these two major proteins, so that any effort to remove these prevalent proteins to maximize an analytical signal from the trace substances is likely to also eliminate the potential biomarkers. In addition, many of the potential biomarkers are likely to be proteins, but enzymes present in blood begin degrading these proteins almost immediately after blood is removed from body.
Nanoparticles can be modified to render surfaces selective for targeted molecular interactions. As the biomarker populations present in blood will be more fully characterized, nanoparticle harvesting platforms will have significant potential for improving disease detection at an early, more treatable stage.

Nano-biopsy
Brain tumours are often the hardest cancers to diagnose in the human body. For diagnosis in other tissues, biopsies allow to determine whether a tumour is benign or malignant. But removing brain tissue should be avoided due to the specificity of this organ. However, the decreased invasiveness enabled by nanotechnology offer an alternative. In order to map brain tumours, a novel technique based on a nano patterned pen has been developed to collect proteins and cells by surface adhesion. This is done through the use of an endoscopic pen which is inserted "through" the brain. Then a small amount of "floating" cells and biomolecules is removed from the target area in the brain, without removing brain tissue. However, because of the extremely delicate nature of the brain, precise placement of the tip of the pen by stereotaxic methods is vital to ensure the sample is removed without causing harm to potentially healthy tissue surrounding it.

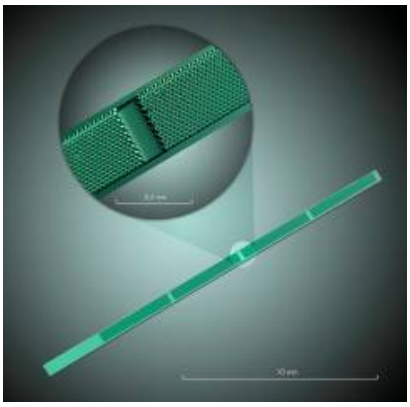

Fig 5: Protool, harvesting device for collecting cells and biomolecules in the brain. Credit: CEA-Leti

6 NanoPharmaceuticals

The scope of pharmacy practice includes more traditional roles such as compounding and dispensing pharmaceutical drugs. Most of pathologies are treated by dispensing drugs. Some of them are small chemical molecules while others are biological ones. However the use of systemic drug administration may generate some side effects. Therefore improvements of drug administration, especially for injectable drugs, are looked for by pharma industry and by patients. Encapsulation of drugs in carriers is a possibility, which has been explored for several tens of years. Nanotechnology offers means to aim therapies directly and selectively at diseased tissues or cells, with application in cancer or

inflammation for instance. The behaviour of nanomaterials used for in vivo administration should be demonstrated whether they are biocompatible, or biodegradable.

6.1 Nanoparticulate Drug Delivery Systems

In the short and medium term, the main use of nanoparticle medicinal products (NMP) is vectorization of active principles, corresponding to several products already marketed like Doxil™. or more recently Abraxane™.
Generally three vector generations are considered:

- First generation vectors: nanospheres and nanocapsules (the best known and most accessible),
- Second generation vectors: nanoparticles coated with hydrophilic polymers such as polyethylene glycol (PEG), PEGylated nanoparticles
- Third generation vectors, still under development, combining a biodegradable core and a polymer envelope (PEG) with a membrane recognition ligand.

Today, most current research projects in nano delivery systems are focused on the third type.

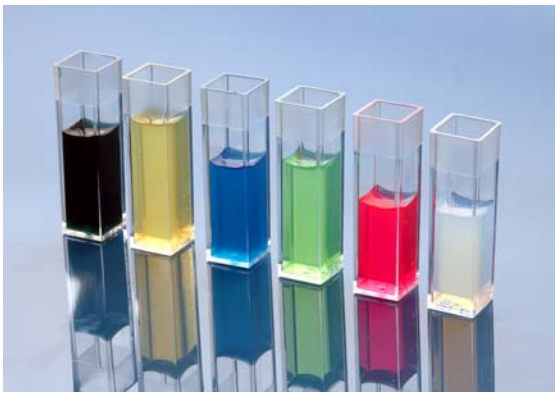

Fig 6 : Lipidots® lipid nanoparticles containing various organic dyes for molecular imaging. Credit: CEA-Leti

Conventional chemotherapy employs drugs that are known to kill cancer cells effectively. But these cytotoxic drugs kill healthy cells in addition to tumor cells, leading to adverse side effects such as nausea, neuropathy, hair-loss, fatigue, and compromised immune function. Nanoparticles can be used as drug carriers for chemotherapeutics to deliver medication directly to the tumor while sparing healthy tissue[9]. Nanocarriers present several advantages over conventional chemotherapy. They can:

- Protect drugs from being degraded in the body before they reach their target.
- Enhance drug absorption into tumors and the cancerous cells themselves.
- Allow for better control over the timing and distribution of drugs to the tissue, making it easier for oncologists to assess how well they work.
- Prevent drugs from interacting with normal cells, thus avoiding side effects.

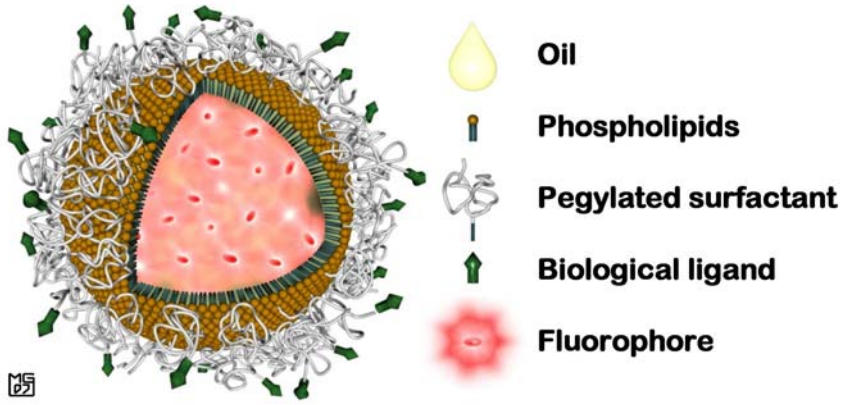

Fig 7: Lipidots® for drug delivery and molecular imaging. Credit: CEA-Leti

Passive Targeting

There are now several nanocarrier-based drugs on the market, which rely on passive targeting through a process known as "enhanced permeability and retention." Because of their size and surface properties, certain nanoparticles can escape through blood vessel walls into tissues. In addition, tumors tend to have leaky blood vessels and defective lymphatic drainage, causing nanoparticles to accumulate in them, thereby concentrating the attached cytotoxic drug where it's needed, protecting healthy tissue and greatly reducing adverse side effects.

Another strategy for passive targeting consists in using myeloid cells like macrophages which absorb nanoparticles and concentrate them in the site to be treated, like a Trojan horse.

Active Targeting

On the horizon are nanoparticles that will actively target drugs to cancerous cells, based on the molecules that they express on their surface. Molecules that bind particular cellular receptors can be attached to a nanoparticle so that it specifically targets cells expressing this receptor. Active targeting can even be used to bring drugs into the cancerous cell, by inducing the cell to absorb the nanocarrier. Active targeting can be combined with passive targeting to further reduce interaction of carried drugs with healthy tissue. Nanotechnology-enabled active and passive targeting can also increase the efficiency of a chemotherapeutic, achieving more significant tumor reduction with lower drug doses.

Destruction from within

Moving away from conventional chemotherapeutic agents that activate normal molecular mechanisms to induce cell death, researchers are exploring ways to physically destroy cancerous cells from within. One such technology—nanoshells—is being used in laboratory to thermally destroy tumors from the inside. Nanoshells can be designed to absorb light at different wavelengths, generating heat (hyperthermia). Once the cancer cells take up the nanoshells (via active targeting), scientists apply near-infrared light that is absorbed by the nanoshells, creating an intense heat inside the tumor that selectively kills tumor cells without disturbing neighbouring healthy cells. Similarly, new targeted magnetic nanoparticles are in development that will both be visible through Magnetic Resonance Imaging (MRI) and can also destroy cells by hyperthermia.

6.2 Drug Delivery (mechanical) Devices

Implanted drug delivery devices – DDD – can take benefit of nanotechnology. Examples are DebioStar or Nanopump™, fabricated by the Swiss company Debiotech[10]. The Nanopump™ is a miniaturized drug delivery pump based on MEMS chips which can be implanted for accurate dosing of various therapeutic compounds with dedicated delivery profiles. The Nanopump™ has been tested for instance for insulin delivery. The precision of nanofabrication and micro techniques enable design and fabrication of ultra small devices with reservoirs, actuators, pumps to control accurately the release of pharmaceutical ingredients. Some parts of these micro systems are at the nanoscale. Due to their small size and low invasiveness, these drug delivery devices can be implanted within the body, even in the brain.

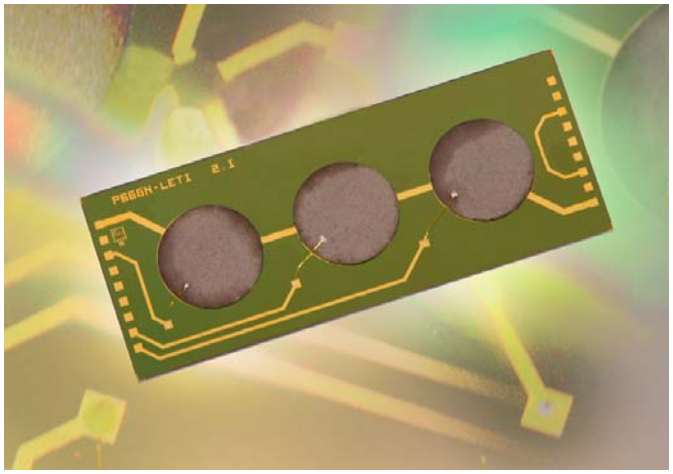

Fig 8: DELICE™miniaturised pump technology for drug delivery. Credit: CEA-Leti

6.3 Theranostics, combined techniques

Nanobiotechnology offers significant inputs to the improvement of detection devices and of the tagging of disease indicators administered in-vivo, which will lead to advancement in imaging. Potent driving forces include synergies, such as those between in-vitro diagnostics (probes and markers) and in-vivo imaging techniques and those between contrast agents / probe development (in drug delivery and/or toxicology studies) and imaging technology (medical instrumentation). The combination of in-vitro diagnostics techniques and in-vivo nano-imaging could lead to targeted tumor disruption or removal: Tagging tumor cells with functionalized nanoparticles, which react to external stimuli, allows for in-situ, localized 'surgery' (breaking up or heating of particles by laser, magnetic fields, microwaves, etc.) without invasiveness within the human body.

The capacity of some nanoparticles to carry contrast agents and drugs opens new ways for therapy. Theranostic, used as a combination of therapy and diagnostics, can be envisaged differently. Imaging can be used to trace the delivery of drug within the body. But imaging can also be used to activate the drug release from outside, by an external stimulus,. Such external stimuli can be laser light, temperature or ultrasounds for instance. All in all, the smart probes represent new concepts for clinical practice.

7 Regenerative medicine

Regenerative medicine is the process of creating living, functional tissues, to repair or replace tissue or organ function lost due to age, disease, damage, or congenital defects. This field holds the promise of regenerating damaged tissues and organs in the body by stimulating previously irreparable organs to heal by themselves. Regenerative medicine could also empowers physicians to grow tissues and organs in laboratory and safely implant them when the body cannot heal by itself[11]. Regeneration of tissues can be achieved by the combination of living cells, which will provide biological functionality, and materials, which act as scaffolds to support cell proliferation.

The vision for nano-assisted regenerative medicine is the development of cost-effective disease-modifying therapies that will allow for in-situ tissue regeneration. The implementation of this approach involves not only a deeper understanding of the basic biology of tissue regeneration – wound healing, in its widest sense – but also the development of effective strategies and tools to initiate and control the regenerative process.

7.1 Stem cells

Combinatorial extracellular matrix micro-nanoarrays, generated by soft-lithography, have great potential in studying and controlling the behaviour of stem cells. Nanomaterial-based gene delivery for manipulating stem cells has a vital role in recognizing the potential of regenerative medicine[12].

The major goal of ongoing and future efforts in regenerative medicine will be to effectively exploit the enormous newly discovered self-repair potential that has been observed in adult stem cells. Given the logistical complexities and the costs associated with today's tissue engineering therapies, which are based on the autologous reimplantation of culture-expanded differentiated cells, next generation therapies will need to build on the progress made with tissue engineering in understanding the huge potential for cell-based therapies which involve undifferentiated cells. Nanotechnology will aid in pursuing two main objectives:

1. Identifying signalling systems in order to leverage the self-healing potential of endogenous adult stem cells
2. Developing efficient targeting systems for adult stem cell therapies.

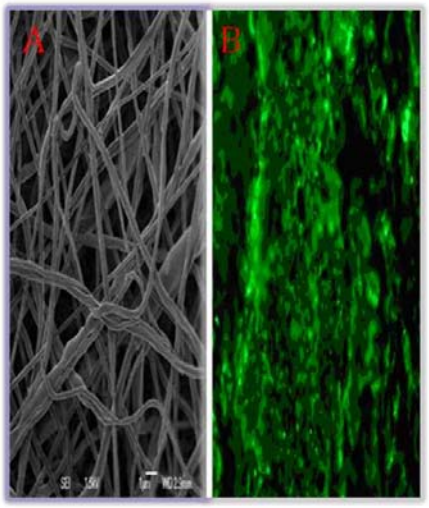

Fig 9: Figure from N. Jessel INSERM U977, Strasbourg, France:

A. A. Manufacture of the (Poly(ε-caprolactone) PCL membrane by electrospining; scale bar 1 μm
B. *B. In vivo bone induction :* Osteopontin expression (in green) on PCL membrane Scale bar: 20 μm.

One possible application for future regenerative medicine strategies is to avoid having to pre-seed a nanostructured biomaterial scaffold or matrix with the patient's own cells, but rather to have the biomaterials loaded with essential signalling molecules targeting adult progenitor cells in the implant site. Thus, understanding how adult human stem cells react to such nanostructures depending on the site of tissue regeneration will be a condition *sine qua non* for specific applications. Cell-based therapies should be aimed at efficient harvesting of adult stem cells, to allow for a brief pre-implantation, cultivation stage, or, preferably, for immediate intra-operative administration using an intelligent biomaterial as a bio-interactive delivery vehicle. Of huge impact would also be the ability to implant cell-free intelligent, bioactive materials that would effectively provide signalling to leverage the self-healing potential of the patient's own stem cells[13].

7.2 Biomaterials

Mammalian cells behave in vivo in response to the biological signals they receive from the surrounding environment, which is structured by nanometre-scaled components. Therefore, materials used in repairing the human body have to reproduce the correct signals that guide the cells towards a desirable behaviour. Nanotechnology is not only an excellent tool to produce material structures that mimic the biological ones but also holds the promise of providing efficient delivery systems. The application of nanotechnology to regenerative medicine is a wide topic. It covers the fabrication of materials, such as nanoparticles and scaffolds for tissue engineering, and surface nanopatterning to elicit specific biological responses from the host tissue[14].

Future biomaterials must simultaneously enhance tissue regeneration while minimizing immune responses and inhibiting infection. While promoters of tissue engineering promised to develop materials that can trigger tissue regeneration for the entire body, such promises have yet not become reality. However, tissue engineering experienced recently great

progress due to the emergence of nanotechnology. Specifically, it has now been well established that enhanced tissue regeneration can be achieved on almost any surface by employing novel nano-textured surface features. Numerous studies have reported that use of nanotechnology allows to accelerate various regenerative therapies, such as those for the bone, vascular, heart, cartilage, bladder and brain tissue. Various nano-structured polymers and metals (alloys) have been investigated for their bio (and cyto) compatibility properties[15].

To apply nanotechnology to stem cell biology several conditions must be fulfilled: nanomaterials must be designed to interact with proteins and cells without perturbing their biological activities; nanomaterials must maintain their physical properties after the surface conjugation chemistry; and nanomaterials must be biocompatible and non toxic[16].

Access to nanotechnology has offered a completely new perspective to the material scientist to mimic the different types of extra-cellular matrices present in tissues. Techniques are now available which can produce macromolecular structures of nanometre size, with finely controlled composition and architecture. Conventional polymer chemistry, combined with novel methodologies such as electrospinning, phase separation, direct patterning and self-assembly, have been used to manufacture a range of structures, such as nanofibres of different and well defined diameters and various surface morphologies, nanofibrous and porous scaffolds, nanowires and nanoguides, nanospheres, nano 'trees' (e.g. dendrimers), nano-composites and other macromolecular structures
In conclusion, nanotechnology can assist in the development of biomimetic, intelligent biomaterials, which are designed to positively react to changes in their immediate environment and stimulate specific regenerative events at the molecular level in order to generate healthy tissues.

8 Ethics

Nanotechnology offers great promise for medicine, but much of this lies in the future. This future orientation has made nanotechnology vulnerable to the current trend of overclaiming in science, either the potential benefit or harm. There is a need to be careful about placing premature weight on speculative hopes or concerns about nanotechnologies raised ahead of evidence. Foresighting of breakthrough technologies is notoriously difficult, and carries the risk that early public engagement may promote either public assurance or concern over wrong issues.
Nanotechnology as an enabling technology for many future medical applications raises issues such as sensitivity of genetic information, the gap between diagnosis and therapy, health care resources and tensions between holistic and functional medicine. As well, nanotechnology will add a new possibilities at the interface between bio (human) and non-bio (machine) such as brain chips or implants, which may eventually raise new ethical issues specific to nanomedicine. This requires careful analysis of ethical aspects in view of existing standards and regulations set by ethics committees at the European scale.
At the same time, new nanomedical inventions have to be evaluated with respect to new ethical aspects by ethical, legal and social aspects - specialists. The key point in this regard is an early proactive analysis of new technological developments to identify and discuss possible issues as soon as possible. This requires a close collaboration and co-learning of technology developers and ethics specialists assisted by communication experts to ensure

open and efficient information of the public about ethical aspects related to nanomedicine. This co-evolution will ensure a socially and ethically accepted development of innovative diagnostic and therapeutic tools in nanomedicine.
From the above it is clear that an in-depth ethical analysis is necessary in this field. Such an analysis should be based on the following principles.

Human Dignity and the derived ethical principles of:

- Non-instrumentalisation: The ethical requirement of not using individuals merely as a means but always as an end of their own.
- Privacy: The ethical principle of not invading a person's right to privacy.
- Non-discrimination: People deserve equal treatment, unless there are reasons that justify difference in treatment. It is a widely accepted principle and in this context it primarily relates to the distribution of health care resources.
- Informed Consent: The ethical principle that patients are not exposed to treatment or research without their free and informed consent.
- Equity: The ethical principle that everybody should have fair access to the benefits under consideration.
- The Precautionary Principle: This principle entails the moral duty of continuous risk assessment with regard to the not fully foreseeable impact of new technologies as in the case of ICT implants in the human body.

The last of these principles (the Precautionary Principle) is particularly important in this particular context.
The ethical analysis should also deal with value conflicts. There could exists conflict between personal freedom to use one's economic resources to obtain advanced treatment such as nanomedicine and what society at large considers desirable or ethically acceptable. Concern for economic competitiveness and other economic values (economic growth) may come into conflict with respect for human dignity. Unrestricted freedom of some may endanger health and safety of others. Therefore a balance has to be struck between values that are all legitimate in our culture.

9 Regulation, approval

European Medicines Agency
The current regulatory framework based on benefit/risk approach and including risk management plan and environmental risk assessment, is adequate for the current development and evaluation of current "nano" application in pharmaceuticals.
Current regulatory experience allows the assessment of many aspects of nanomedicines, but there is a scientific gap between the current knowledge and the more advanced and emerging nanomedicines. This gap is an opportunity for scientific research[17]. It is expected that new methods will be implemented to complement the relevant existing guidelines and new features will be assessed as they emerge.

Agence Française de Sécurité Sanitaire des Produits de Santé – Working Group on non clinical innovation

AFSSAPS[1] has released in 2009 a position paper approved by the Experts of the Reflexion Group on new orientations on evaluating non clinical safety of Health products[2]. According to this expert group3, and as indicated above, "*...general scientific and/or regulatory data related to the toxicological evaluation of nanoparticle medicinal products (NMP) are currently lacking… However the "conventional" toxicological approach proposed by current guidelines for medicinal products in general has been accepted up until now for approved NMPs or NMPs currently under evaluation by health authorities. However, some criticisms have been raised concerning the currently available methods of experimental evaluation that are considered to not adequately assess the properties of nanoparticle products…… Consequently, like certain consumer groups in the USA, we may need to recommend the development of completely new regulations based on "adapted" safety assessment tests for nanomaterials, including NMPs. This maximalist proposal is totally idealistic and scientifically unjustified according to the very great majority of the scientific community. How many years for development and validation would be necessary to achieve such a result? This major revision also does not appear to be justified by the available scientific data. Some manufacturers and most of the AFSSAPS task force also consider that toxicological evaluation of NMPs should not be appreciably different from "conventional" evaluation, but with certain specific adaptations. The plan adopted for elaboration of these recommendations is based on this latter approach, i.e. adapt the safety assessment strategy, when necessary, without modifying the basic principles*"[18].

US Food and Drug Administration (FDA)

According to FDA : "*Nanomedicine is really no different than any other new technology that would be incorporated into FDA products. So with that in mind, we feel comfortable using our present regulatory framework. However, we felt there is need for guidance to help this industry as it moves forward. We recognized a need for additional information in various areas, such as biosafety. FDA and other agencies are working together on that. But for now, we just do not see the need for regulations written specifically for nano-engineered materials in the products FDA regulates*"(2008)[19].

The existing regulatory framework can accommodate the types of nanoparticle therapeutics under development and when needed, adapt to address new challenges. Current published guidance may be applicable to nanoparticle therapeutics. Staff is working on addressing the need for guidance documents that address nano-related issues as well as the regulatory science to bring to bear this emerging technology (2010)[20]".

10 The industrial perspective

Nanomedicine is no longer restricted to an academic concept. That is now also an industrial reality, a market with approved products and devices. Nanomedicine is a large industry, with sales reaching 6.8 billion dollars in 2004, and with over 200 companies and 38 products worldwide. A minimum of 3.8 billion dollars in nanotechnology R&D is being invested every year.[1] As the nanomedicine industry keeps on growing, it is expected to significantly impact economy.

[1] Agence Française de Sécurité Sanitaire des Produits de Santé (French Health Products Safety Agency)

[2] Groupe de Réflexion sur les Nouvelles Orientations en Matière d'Évaluation Non-clinique de la Sécurité des Produits de Santé de l'Afssaps

Trade name	company	Year approved	Ative pharmaceutical ingredient	Indication	Nanotechnology
Visudyne		2000	Verteporfin	Photodynamlic therapy for age-related macular degeneration	Liposome
Doxil/Caelyx		1995	Doxorubicin	Antineoplastic	PEGylated liposome
AmBisome		1990	Amphotericin B	Fungal infections	Liposome
Abelcet		1995	Amphotericin B	Fungal infections	Liposome
Definity		2001	Octofluoro-propane		Liposome
Myocet		2001	Doxorubicin		Liposome
DepoCyte		1999	Cytarabine	Lymphomatous meningitis	Liposome
DepoDur		2004	Morphine		Liposome
Daunoxome		1996	Daunirubicin	Antineoplastic	Liposome
Octocog alfa		2009	Factor VIII		Liposome
Abraxane	Abraxis Biosciences	2005	paclitaxel	Metastatic breast cancer	Albumin bound nanoparticles
Rapamune	Wyeth	2000	Rapamycin	Immunosuppresant	Nanocrystal Elan
Emend	Merck	2003	Aprepitant	Anti-emetic	Nanocrystal Elan
Tricor	Abbott	2004	Fenofibrate	Hypercholesterolemia	Nanocrystal Elan
Megace ES	Par Pharma Co	2005	Megestrol	Anti-anoretic	Nanocrystal Elan
Triglide	Sciele Pharma Inc.	2005	Fenofibrate	Hypercholesterolemia	IDP-P Skyepharma nanocrystal
Mepact			Mifamurtide		Liposome
Amphotec		1996		Fungal infections	Micelle
Estrasorb		2003		Vasomotor symptoms associated with menopause	Micelle
Taxotere		1996		Antineoplastic	Micelle
Somatuline depot		2007		Acromegaly	Nanotube
Feraheme injection		2009		Treatment of iron deficiency anemia in patient with Chronic Kidney Disease	SPIO

Table 14: List of drugs approved by FDA. Source: Adapted from US-FDA and EMA

Only a few studies provide clear estimates of nanomedicine market., Drug delivery represents the largest application area, while the biomaterials are the fastest-growing application segment over the years 2006 through 2015. However the nanomedicine market is highly fragmented and is characterized by the presence of several niche players. The following players are among the largest ones or most active ones in nanomedicine:

- Nanosphere Inc.
- Par Pharmaceutical Companies Inc.
- AMAG Pharmaceutical Inc.
- Elan Corporation PLC
- Life Technologies Corporation
- Abraxis BioScience Inc. (now CelGene)
- Flamel Technologies S.A.

- Oxonica Plc
- Wyeth Pharmaceuticals Inc
- MagForce GmbH
- NanoBiotix
- Arrowhead Research Corporation[21]
- …

The global nanomedicine market was valued at $53 billion in 2009, and is forecast to increase at a compound annual growth rate (CAGR) of 13.5% to reach more than $100 billion in 2014. Nanomedical products for cancer are one of the largest market segments, worth nearly $20 billion in 2009. This sector is expected to increase at a compound annual growth rate (CAGR) of 11% to reach $33 billion in 2014. Nanomedicine for central nervous system related indications is another major market sector, valued at nearly $11 billion in 2009 and expected to reach $18 billion by 2014, an 11.1% compound annual growth rate (CAGR).[22]

Although the number of nanotech based medicines may look limited, a significant number of clinical trials involving the use of nanoparticles, 87 precisely, are listed in the US NIH data base www.clinicaltrials.gov , especially in phase I and phase II. It is expected that some if them will reach the approval stage and then the market in a few years, thus increasing the number of successful nanopharmaceuticals.

The dominant model for the innovation chain in nanobiotech is of type A where discovery made in academic labs is transferred to spin-off or high tech SMEs addressing niche markets, validating the concept and initiating the first clinical trials, sometimes up to stage IIa. Then large companies take over these SMEs with innovative and validated devices, molecules or concepts.

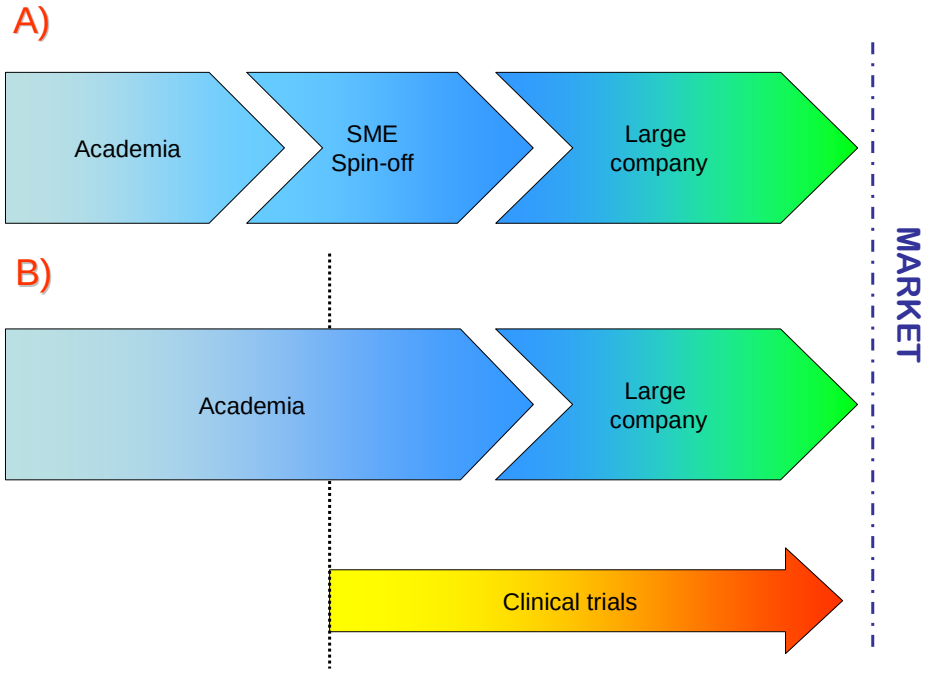

Fig 10: innovation chain of nanomedicine.Credit: personal data

The Nanomedicine European Technology Platform (ETP) established in 2005 is an initiative led by industry and set up together with the European Commission, addressing the application of nanotechnology to achieve breakthroughs in healthcare. The ETP supports its members in coordinating their joint research efforts and improving communication amongst them as well as towards the European Commission and the European Member States. The Nanomedicine ETP promotes the development of the nanomedicine industry in Europe, in liaising industry, academia and hospitals for translating nanomedicine research "from bench to bedside". Its role becomes even more important in the context of open innovation.

Nanomedicine is a recent set of technologies. Research significantly started about ten years ago only. It is usually considered that the average development time for any high tech ranges from 10 to 15 years. Therefore nanomedicine is not developing more slowly than other high technology. Some large companies are however sometimes reluctant to invest into nanomedicine R&D projects because there are still some uncertainties about the regulation to be applied to this kind of techniques. In addition, poor social acceptance of nanotechnologies in general, and opposition expressed in some countries against inorganic nanoparticles is not favourable to investment, even though nanomedicine is much better accepted by the general public than use of nanotechnologies in manufactured products. The example of the French public debate on nanotechnology in 2009-2010 highlights this point of view[23].

11 French stakeholders in nanomedicine

Nanomedicine is an active area in the French academia and in the small business community. Since the early 2000's, research institutes, universities, some hospitals and a few years later, SMEs and some large companies have shown strong interest in nanomedicine by participating to various European and national initiatives. It can be said that today that all stakeholder are involved.

Ministries

Nanotechnology has been considered for many years as a strategic priority by the French Ministries of Research and Industry. Research in nanotechnology is a strategic priority in some universities and several research organisations. Since 2003, a network of nanotech facilities has been supported by French government to facilitate access of academia or industry to nanotechnology. More recently, in May 2009, an additional effort to support tech transfer to industry has been invested by the Ministry of Research by investing 70 millions Euros in three major centres: Saclay, Grenoble and Toulouse.

Research in nanosciences and nanotechnology in France represents approximately 5300 scientists and 243 labs. France is ranked at the 2nd or 3rd position in Europe by its number of publications while it is ranked 6th in the world behind the United Kingdom, Germany and China.

A public debate has been organised in late 2009 and 2010 about public acceptance and perception of nanotechnology[24]. It had three objectives:

1. Inform the public about nanosciences and nanotechnology
2. Listen to questions, reactions, statement by the public
3. Publish all what has been discussed and presented over the four months period.

It's important to note that while there was some strong opposition to the use of nanotechnologies in products like food, or manufactured products, nanomedicine is much better perceived. Nanomedicine, even if it leads to the use some nano components, is considered as part of the medical progress and research should be pursued, in conformity with the legal and regulatory framework.

AVIESAN ITMO-TS

Set up in April 2009, the French National Alliance for Life Sciences and Health (Aviesan) groups together the main stakeholders of life and health sciences in France. AVIESAN is considered as the organisation where academia and clinicians are best represented to discuss about research. Among the ten thematic institutes of this Alliance, the Institute for Health Technologies coordinates research in the field of technologies that are essential to biomedical progress in both fundamental and clinical terms. As such, it started a working group dedicated to nanomedicine in 2010.

Companies

There is no dedicated database or directory of French companies involved in nanomedicine. In fact, very few companies consider themselves as "nanomedicine companies" with some exception like Nanobiotix, developing NanoXray®. Nanomedicine is rather considered as an advanced medical technology. Therefore few companies are labelling their product "nano" even if it fits with the definition of nanotechnology, like Abraxane® for instance.
However, the data base Biotechnology in France[25] lists approximately 50 companies but much less should be really considered as nanomedicine companies like:

- BioAlliance Pharma
- BioMérieux
- Carlina Technologies
- Cezanne
- Cytoo
- Diatos
- Etypharm
- Flamel Technologies
- Fluoptics
- Genoptics
- Guerbet
- Imstar
- InoDiag
- Medsqual
- Nano-H
- NanoBioTix
- … (*non exhaustive list*)

Agencies

The French Medicine Agency AFSSAPS is responsible for the clearance of nanomedicine products like any other drug or medical device. A working group from AFSSAPS released in 2009 recommendations for toxicological evaluation of nanoparticle medicinal products.

The French National Research Agency ANR is supporting nanomedicine related research in several funding programmes. Except in the 2010-2011 programme *Investissements d'Avenir* (French National recovery plan), no funding programme is fully dedicated to nanobiotechnology and nanomedicine as such. However, nanomedicine related topics are eligible and supported under several programmes like pNano, BiotecS, TecSan, Emergence…. The French National Research Agency ANR is also supporting the French labs participating to ERANET EuroNanoMed projects, from 2010.

12 Conclusion

Like most high technologies and taking into account the high regulation of medical sector, it takes 10-15 years to any advanced medical technology before reaching the market. Considering significant efforts put in nanomedicine related basic research in the early 2000's, it is expected that a significant number of approved nanodrugs or nanodevices will be approved in the early 2010's. So far 22 nanodrugs are approved by FDA, and many more are under clinical trials phase II or even III. The same situation occurs in Europe with approximately 25 on going clinical trials in 2010[26]. In the same time, more SMEs and spin-off companies target medical applications using nanotechnologies, sometimes niche markets, contributing to the validation of innovative concepts. It is easy forecasting that large companies, like pharma, imaging or diagnostics companies will help some of the most promising companies to reach the market. Then we will see what the real medical applications are for which nanotechnology brings a real added value in a cost effective manner.

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